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     (FILE 'HOME' ENTERED AT 21:31:38 ON 28 JAN 2003)

     FILE 'CAPLUS, USPATFULL' ENTERED AT 21:31:49 ON 28 JAN 2003
L1            52 FILE CAPLUS
L2           110 FILE USPATFULL
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L3           162 S FIBROBLAST (1S) WRINKLE
L4             1 FILE CAPLUS
L5             5 FILE USPATFULL
     TOTAL FOR ALL FILES
L6             6 S EXCESSIVE (2S) L3
L7             0 FILE CAPLUS
L8            12 FILE USPATFULL
     TOTAL FOR ALL FILES
L9            12 S WRINKLE/CLM AND FIBROBLAST/CLM
L10           66 FILE CAPLUS
L11           11 FILE USPATFULL
     TOTAL FOR ALL FILES
L12           77 S WRINKLE/AB AND FIBROBLAST/AB
L13            2 FILE CAPLUS
L14            0 FILE USPATFULL
     TOTAL FOR ALL FILES
L15            2 S EXCESS?/AB AND L12
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     TOTAL FOR ALL FILES
L21           57 S FIBROBLAST? (2S) TREAT? (2S) WRINKLE
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     TOTAL FOR ALL FILES
L24          444 S FIBROBLAST? (2S) (SKIN OR TISSUE) (2S) (RELAX? OR LOOSE?)
L25            0 FILE CAPLUS
L26            4 FILE USPATFULL
     TOTAL FOR ALL FILES
L27            4 S L24 (3S) WRINKLE

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  75% OF LIMIT FOR SAVED L# LISTS REACHED
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L39 ANSWER 1 OF 32 CAPLUS COPYRIGHT 2003 ACS
AN 1998:344907 CAPLUS
DN 129:113499
TI The healing process of palatal tissues after palatal surgery with and without implantation of membranes: an experimental study in dogs
AU Leenstra, T. S.; Kuijpers-Jagtman, A. M.; Maltha, J. C.
CS Dep. Orthodontics and Oral Biology, Univ. Nijmegen and Cleft Palate Center, Univ. Hospital, Nijmegen, 6500 HB, Neth.
SO Journal of Materials Science: Materials in Medicine (1998), 9(5), 249-255
CODEN: JSMMEL; ISSN: 0957-4530
PB Chapman & Hall
DT Journal
LA English
CC 63-7 (Pharmaceuticals)
AB The aim of this study was to evaluate the wound-healing process clin. and histol. in growing beagle dogs after palatal repair according to von Langenbeck, with and without implantation of membranes of a copolymer of polyhydroxybutyrate 80%-hydroxyvalerate 20% (=PHB-co-HV 80/20). Von Langenbeck's repair was performed in 12 dogs (age 12 wk), while von Langenbeck's repair followed by implantation of PHV-co-HV membranes was carried out in 11 dogs (age 12 wk). Four dogs (age 12 wk) served as unoperated controls. Standardized intra-oral slides of the palate where taken and measurements of the wound surface areas were carried out. Histol. sections were prepd. at three different age. The animals were studied until the age of 25 wk. It was found that **wound** closure after the von Langenbeck's procedure took about 3 wk, while the use of PHB-co-HV membranes after von Langenbeck's repair resulted in complete **wound** closure after approx. 7 wk after the membranes had sequestered. At the age of 25 wk, the histol. results after the von Langenbeck procedure showed that the entire **scar tissue** covering the former denuded bony areas was attached to the bone by means of Sharpey's fibers, while after implantation of the membranes only local **scar tissue** attachment by means of Sharpey's fibers was found. Further research is necessary to develop a membrane which allows **wound** closure without sequestration of it.
ST palate wound healing polymer membrane implant
IT Palate
Wound healing
(healing of palatal tissues after palatal surgery with and without implantation of membranes)
IT Polyesters, biological studies
RL: ADV (Adverse effect, including toxicity); BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); DEV (Device component use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(**hydroxy acid**-based; healing of palatal tissues after palatal surgery with and without implantation of membranes)
IT Dental materials and appliances
Prosthetic materials and Prosthetics
(implants; healing of palatal tissues after palatal surgery with and without implantation of membranes)
IT 80181-31-3 128171-16-4, Hydroxybutyric acid-hydroxyvaleric acid copolymer
RL: ADV (Adverse effect, including toxicity); BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); DEV (Device component use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(healing of palatal tissues after palatal surgery with and without implantation of membranes)
RE.CNT 17 THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE

(1) Baier, R; Biomaterials 1982, V3, P241 CAPLUS
(2) de Braekt, M; Cleft Palate Craniofac J 1993, V30, P129
(3) de Braekt, M; Cleft Palate Craniofac J 1995, V32, P290
(4) de Braekt, M; J Oral Maxillofac Surg 1992, V50, P359
(5) Den Braber, E; J Biomed Mater Res 1995, V29, P511 CAPLUS
(6) Densho, S; Sapporo Med J 1982, V51, P243
(7) Ham, A; Histology 1979, P387
(8) Herfert, O; Dtsch Zahn-Mund-Kieferheilk 1954, V20, P369
(9) Herfert, O; Dtsch Zahn-Mund-Kieferheilk 1956, V24, P112
(10) Herfert, O; Dtsch Zahn-Mund-Kieferheilk 1958, V11, P97
(11) Jansen, J; Biomaterials 1991, V12, P25 CAPLUS
(12) Kremenak, C; Cleft Palate J 1967, V4, P6 MEDLINE
(13) Kremenak, C; Cleft Palate J 1970, V7, P719
(14) Kremenak, C; Otolaryngol Clin North Am 1984, V17, P437 MEDLINE
(15) Leenstra, T; J Mater Sci Mater Med 1995, V6, P445 CAPLUS
(16) Wijdeveld, M; Arch Oral Biol 1991, V36, P837 MEDLINE
(17) Wijdeveld, M; J Dent Res 1989, V68, P1105 MEDLINE

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L27 ANSWER 1 OF 4 USPATFULL

DETD In addition to the treatment methods discussed herein, in other embodiments the invention can be configured for **skin** rejuvenation. In these embodiments, the delivery of thermal energy to the target **tissue** is controlled/reduce to only cause a wound healing response and not necessarily collagen contraction. This would healing response results by delivering thermal energy to the **tissue** to induce a condition called fibroplasia. This is a condition in which there is a proliferation or otherwise infiltration into the dermis of a large number of **fibroblast** cells. These **fibroblast** cells in turn, lay down or deposit collagen into or adjacent the thermal affect zone causing the **skin** rejuvenation process. However by delivering a selected amount of energy, a proportion of the **fibroblasts** in the dermis can be killed off. As a result, a wound healing response occurs, in which there is large infiltration of **fibroblasts** into the dermis, with a large number of **fibroblasts** present than before treatment. These new **fibroblasts** lay down new collagen as part of a wound healing response and this rejuvenates the **skin**. Thus by controlling the amount of thermal energy delivery to the target **tissue** (and/or temperature of), the resulting **tissue** affect can be titrated to produce **skin** rejuvenation for lower levels of delivered energy, or collagen contraction configured to tighten the **skin** for higher levels of delivered energy. If the collagen contraction/**skin** tightening is positioned very superficially, it can help to minimize the appearance of **wrinkles**. If the area of collagen contraction is located deeper in the dermis, it can tighten up areas of **loose skin**.

ACCESSION NUMBER:	2002:160137 USPATFULL
TITLE:	Apparatus and method for treatment of tissue
INVENTOR(S):	Stern, Roger A., Cupertino, CA, United States
PATENT ASSIGNEE(S):	Thermage, Inc., Hayward, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6413255	B1	20020702	
APPLICATION INFO.:	US 2000-522275		20000309	(9)

	NUMBER	DATE	
PRIORITY INFORMATION:	US 1999-123440P	19990309	(60)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	GRANTED

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     (FILE 'HOME' ENTERED AT 21:31:38 ON 28 JAN 2003)

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L2           110 FILE USPATFULL
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L3           162 S FIBROBLAST (1S) WRINKLE
L4             1 FILE CAPLUS
L5             5 FILE USPATFULL
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L6             6 S EXCESSIVE (2S) L3
L7             0 FILE CAPLUS
L8            12 FILE USPATFULL
     TOTAL FOR ALL FILES
L9            12 S WRINKLE/CLM AND FIBROBLAST/CLM
L10           66 FILE CAPLUS
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     TOTAL FOR ALL FILES
L12           77 S WRINKLE/AB AND FIBROBLAST/AB
L13            2 FILE CAPLUS
L14            0 FILE USPATFULL
     TOTAL FOR ALL FILES
L15            2 S EXCESS?/AB AND L12
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L21 ANSWER 18 OF 57 CAPLUS COPYRIGHT 2003 ACS
AN 1993:479748 CAPLUS
DN 119:79748
TI Approach to the **treatment** of **wrinkle** with cosmetics. Investigations using cultured human dermal **fibroblasts**
AU Tanaka, Hiroshi; Nagase, Kenichi; Okada, Tomio
CS Biochem. Res. Inst., Nippon Menard Cosmet. Co., Ltd., Ogaki, 503, Japan
SO Nippon Koshohin Kagakkaishi (1992), 16(3), 182-5
CODEN: NKKAEV; ISSN: 0287-1238
DT Journal; General Review
LA Japanese
CC 62-0 (Essential Oils and Cosmetics)
AB A review, with 20 refs., of the **treatment** of **wrinkle** from aging with cosmetics, using cultured human dermal **fibroblasts** for evaluation of efficacy.
ST review wrinkle aging cosmetic skin fibroblast
IT **Fibroblast**
(cultured dermal, of humans, cosmetics **treatment** of **wrinkle** from aging evaluation by)
IT Cosmetics
(**wrinkle** from aging **treatment** with, cultured human dermal **fibroblasts** for evaluation of)
IT Senescence
(**wrinkle** from, cosmetics **treatment** of, cultured human dermal **fibroblasts** for evaluation of)

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L27 ANSWER 4 OF 4 USPATFULL

SUMM In particular, a surprising activity of the Smelophyllum capense extracts has been discovered on the synthesis of collagen, in particular of type I collagen, hereinafter referred to as the abbreviation "collagen I". Now the **skin** essentially contains collagen I, a protein synthesised by the **fibroblasts** which are the major cells of the dermis. This protein plays a support role and is responsible for the rheological qualities of the dermis, in particular, it is responsible for its firmness and for the upkeep of its structure (E. U. KUCHARZ, "The collagens: Biochemistry and pathophysiology", Springer Verlag, Berlin 1992). Furthermore, it has been demonstrated that the **fibroblasts** of the dermis of elderly people secrete less collagen than those of young subjects (M. DUMAS et al, Mech, Ageing Dev. (1994) 73, 179-187). Thus, with age, a decrease of the rheological qualities, and a decrease in its response to constraints to which it is submitted very day is produced. The **skin** stretches, reacts less well to tensions, **looses** its tonus and **wrinkles** form.

ACCESSION NUMBER:	1998:111648 USPATFULL			
TITLE:	Skin treatments with Smelophyllum capense extracts			
INVENTOR(S):	Bonte, Frederic, Courbevoie, France			
	Dumas, Marc, Colombes, France			
	Lavaud, Catherine, Tinqueux, France			
	Massiot, Georges, Reims, France			
PATENT ASSIGNEE(S):	LVMH Recherche, Nanterre, France (non-U.S. corporation)			

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5807555		19980915	
	WO 9620000		19960704	
APPLICATION INFO.:	US 1997-849453		19970618	(8)
	WO 1995-FR1724		19951222	
			19970618	PCT 371 date
			19970618	PCT 102(e) date

	NUMBER	DATE
PRIORITY INFORMATION:	FR 1994-15576	19941223

DOCUMENT TYPE:	Utility
FILE SEGMENT:	Granted
PRIMARY EXAMINER:	Naff, David M.
ASSISTANT EXAMINER:	Kerr, Janet M.
LEGAL REPRESENTATIVE:	Dennison, Meserole, Pollack & Scheiner
NUMBER OF CLAIMS:	35
EXEMPLARY CLAIM:	1
LINE COUNT:	508

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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L27 ANSWER 3 OF 4 USPATFULL

SUMM Elastase, elastin degradation enzyme, is present in the cells, especially in the dermal cells (**fibroblasts**) just as, in a smaller measure, in the epidermal cells (keratinocytes). It has been observed that the quantity and activity of elastase increases during the cutaneous ageing process, intrinsic as well as actinic. By a degradation of the elastin fibres, the result of the elastase action is a loss of cutaneous elasticity, a **relaxing** of the **skin** and the appearance of **wrinkles**.

ACCESSION NUMBER:	2001:78701 USPATFULL			
TITLE:	Use of an extract of Cordia dichotoma			
INVENTOR(S):	Renimel, Isabelle, Trainou, France Olivier, Marc, Les Angles, France Andre, Patrice, Neuville aux Bois, France Cabalion, Pierre, Noumea, France			
PATENT ASSIGNEE(S):	Parfums Christian Dior, Paris, France (non-U.S. corporation)			
	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6238674	B1	20010529	
	WO 9827957		19980702	
APPLICATION INFO.:	US 1999-319935		19990618	(9)
	WO 1997-FR2343		19971218	
			19990618	PCT 371 date
			19990618	PCT 102(e) date
	NUMBER		DATE	
PRIORITY INFORMATION:	FR 1996-15794		19961220	
DOCUMENT TYPE:	Utility			
FILE SEGMENT:	Granted			
PRIMARY EXAMINER:	Tate, Christopher R.			

L27 ANSWER 2 OF 4 USPATFULL

DETD [0046] In some embodiments, e.g., for the study of aging **skin**, the **fibroblasts** and/or keratinocytes can be senescencing. Senescencing cells can be formed by passing the cells over and over. The number of passages will be dependent on the cell type. In each case, the skilled artisan will recognize when the cells are senescencing. Alternatively, the senescencing cells can be derived from primary sources wherein the individual shows the symptoms of aging **skin** such as **looseness**, dryness and/or **wrinkles**.

ACCESSION NUMBER: 2001:223701 USPATFULL
TITLE: SKIN EQUIVALENT AND METHODS OF FORMING AND USING SAME
INVENTOR(S): HOEFFLER, WARREN, SAN CARLOS, CA, United States
NELSON, CHARLOTTE F., SUISUN, CA, United States
WANG, CHIAOYIN KATHY, PALO ALTO, CA, United States

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2001048917	A1	20011206
APPLICATION INFO.:	US 1998-37191	A1	19980309 (9)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: FLEHR HOHBACH TEST, ALBRITTON & HERBERT LLP, SUITE 3400 FOUR EMBARCADERO CENTER, SAN FRANCISCO, CA, 94111
NUMBER OF CLAIMS: 30
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 7 Drawing Page(s)
LINE COUNT: 1144
CAS INDEXING IS AVAILABLE FOR THIS PA

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     FILE 'CAPLUS' ENTERED AT 20:15:41 ON 28 JAN 2003
L2             1 S US6344461/PN

     FILE 'CAPLUS' ENTERED AT 20:17:35 ON 28 JAN 2003
                 E CALCIUM CHANNEL BLOCKER/CT
                 E E5+ALL
                 E CALCIUM CHANNEL/CT
L3        734180 S E17+ALL
                 E CALCIUM CHANNEL ANTAGONIST/CT

     FILE 'HCAPLUS' ENTERED AT 20:24:28 ON 28 JAN 2003
                 E CALCIUM CHANNEL BLOCKER/CT
                 E E43=ALL
                 E E43+ALL
                 E E41+ALL
L4          23797 S CALCIUM CHANNEL
L5           5665 S WRINKLE
L6              2 S L4 AND L5
L7              0 S CALCIUM CHANNEL BLOCKER/CT
                  E CALCIUM CHANNEL BLOCKER/CT
L8              0 S E80
                  E E80+ALL
L9             96 S E90
L10             0 S L9 AND (WRINKLE OR (FINE LINE?) OR (SKIN (2A) (FIRMING OR BEA
L11             4 S L9 AND (MUSCLE  (1S) (RELAX? OR DECONTRACT? OR LOOSE? OR CONT
L12             0 S L9 AND ( (SKIN (2A) (FIRMING OR BEAUTIFYING)) )
L13             0 S L9 AND ( FACE OR FACIAL)

     FILE 'CAPLUS' ENTERED AT 20:34:06 ON 28 JAN 2003
L14           398 F HID
L15             0 S L9 AND COSMETIC
L16            37 S E90+USE
L17             0 S L16 AND WRINKLE?
L18           120 S CALCIUM AND WRINKLE?
L19             5 S L18 AND CHANNEL
                  E VASODILATOR/CT
                  E E100+ALL
L20        203818 S E106+RT
L21          4032 S E116
L22           719 S L20 (L) CALCIUM CHANNEL
L23             0 S L20 (L) (WRINKLE? OR (FINE LINE))
L24            53 S L20 AND (WRINKLE? OR (FINE LINE))
L25             0 S L24 AND L22
L26            13 S L21 AND (WRINKLE? OR (FINE LINE))
                  SET SMA OFF
                  SEL RAN.CAPLUS(7) L26 5
                  SET SMA LOGIN
L27             1 S E122
L28           547 S VERPAMIL OR ANIPAMIL OR GALLOPAMIL OR DEVAPAMIL OR FALIPAMIL
L29         13602 S NIFEDIPINE OR AMLODIPINE OR DAZODIPINE OR FELODIPINE
L30          3738 S ISRADIPINE OR LANICARDIPINE OR NIMODIPINE OR NISOLDIPINE
L31          9600 S NITRENDIPINE OR TYOSIDINE OR DILTIAZEM OR CINNARIZINE OR FLUN
L32         23242 S L28-L31
L33             1 S L32 (2S) WRINKLE?

     FILE 'JAPIO' ENTERED AT 20:49:17 ON 28 JAN 2003

     FILE 'KOSMET, JAPIO' ENTERED AT 20:50:06 ON 28 JAN 2003
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L34             9 FILE KOSMET
L35           235 FILE JAPIO
     TOTAL FOR ALL FILES
L36           244 S L32 OR (CALCIUM CHANNEL)
L37             0 FILE KOSMET
L38             1 FILE JAPIO
     TOTAL FOR ALL FILES
L39             1 S L36 AND WRINKLE?
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L21 ANSWER 18 OF 42 USPATFULL

AB A transdermal delivery system for the modulated administration of drugs is described. The drug delivery device comprises a backing; a drug reservoir containing the drug, a plasticizer-type enhancer, a solvent-type enhancer, and optionally, a gelling agent; a non-rate-controlling membrane; and an adhesive layer containing a plasticizer-type enhancer. This drug delivery system is particularly useful for the administration of tolerance-inducing drugs, for example, **vasodilators**, such as isosorbide dinitrate.

SUMM A method of delivering a tolerance-inducing drug, and particularly a **vasodilator**, such as isosorbide dinitrate, is also described. This method comprises placing the transdermal delivery system on the skin of a patient in need of the drug and administering the drug, preferably via a three-phase modulated drug delivery pattern, through the patient's skin at a therapeutically effective dose.

DETD The only limitation to the use of this system for a drug for transdermal use is that the drug have at least one form which permeates through the skin and any barriers of the system between the drug and the skin. Examples of types of drugs that can be used in the inventive device include analgesics, anesthetics, antianginals, e.g., **calcium** channel blockers, antifungals, antibiotics, anticancer drugs, antiinflammatories, anthelmintics, antidotes, antiemetics, antihistamines, antihypertensives, antimalarials, antimigraine agents, antimicrobials, antipsychotics, antipyretics, antiseptics, antiarthritics, antithrombin agents, antituberculotics, antitussives, antivirals, appetite suppressants, cardioactive drugs, chemical dependency drugs, cathartics, chemotherapeutic agents, coronary, cerebral, or peripheral **vasodilators**, contraceptive agents, antidepressants, depressants, diagnostic aids, diuretics, expectorants, hormonal agents, hypnotics, immunosuppression agents, narcotic antagonists, parasympathomimetics, sedatives, stimulants, sympathomimetics, tranquilizers, urinary antiinfectives, vasoconstrictors, and the like. The preferred drugs are those which are effective at relatively low concentration in the blood stream.

DETD TABLE I

Tolerance-inducing Drugs	
Antazoline hydrochloride	Triprolidine hydrochloride
Astemizole	Amphotericin B
Azatadine maleate	Imipenem
Bromodiphenhydramine hydrochloride	Cilastatin sodium
	Primaquine phosphate
Brompheniramine maleate	Co-trimoxazole
Carbinoxamine maleate	Sulfamethoxazole
Chlorpheniramine maleate	Trimethoprim
Chlorpheniramine tannate	Pentamidine Isethionate
Clemastine fumarate	Interferon Alfa-2a;
Cyproheptadine hydrochloride	Interferon Alfa-2b;
	Interferon Alfa-2c;
Dexbrompheniramine maleate	Interferon Alfa-n1;
Dexchlorpheniramine maleate	

Interferon Alfa-n3
Diphenhydramine citrate
Trihexyphenidyl
Diphenhydramine Hydrochloride
hydrochloride Bitolterol Mesylate
Doxylamine succinate
Ephedrine Hydrochloride;
Methdilazine hydrochloride
Ephedrine Sulfate
Promethazine hydrochloride
Isoetharine Hydrochloride;
Terfenadine Isoetharine Mesylate
Trimeprazine tartrate
Isoproterenol Hydrochloride;
Tripelennamine citrate
Isoproterenol Sulfate
Tripelennamine hydrochloride
Mephentermine Sulfate
Metaproterenol Sulfate
trihydrate
Metaraminol Bitartrate
Buprenorphine hydrochloride
Methoxamine Hydrochloride
Naltrexone hydrochloride
Phenylephrine Bitartrate;
Fluoxetine hydrochloride
Phenylephrine Hydrochloride
Clozapine
Phenylpropanolamine
Amphetamine sulfate
Hydrochloride Dextroamphetamine sulfate
Atracurium Besylate
Methylphenidate
Gallamine Triethiodide
hydrochloride
Metocurine iodide
Bendroflumethiazide
Pancuronium bromide
Benzthiazide
Succinylcholine chloride
Chlorothiazide sodium
Tubocurarine chloride
Chlorthalidone
Vecuronium bromide
Cyclothiazide
Succinylcholine chloride
hydrochlorothiazide
Nicotine polacrilex
Hydroflumethiazide
Nicotine Methyclothiazide metolazone
Atenolol Polythiazide
Acebutolol hydrochloride
Quinethazone
Captopril Trichlormethiazide
Diltiazem hydrochloride
Indapamide
Enalapril maleate
Bumetanide
Enalaprilat Ethacrynic Acid
Metoprolol tartrate
Ethacrynate Sodium
Nadolol Furosemide
Nifedipine Cocaine hydrochloride
Propranolol hydrochloride

Famotidine
Hydrochlorothiazide
Edetate **calcium** disodium
Timolol maleate Desmopressin acetate
Verapamil hydrochloride
Lypressin
Clonidine hydrochloride
Bupivacaine hydrochloride
Chlorthalidone Chloroprocaine hydrochloride
Guanabenz acetate
Etidocaine hydrochloride
Guanethidine monosulfate
Lidocaine hydrochloride
Guanadrel sulfate
Mepivacaine hydrochloride
Labetalol hydrochloride
Prilocaine hydrochloride
Hydralazine hydrochloride
Procaine hydrochloride
Methyldopate hydrochloride
Propoxycaine hydrochloride
Methyldopa and Tetracaine hydrochloride
Chlorothiazide Alclometasone dipropionate
Methyldopa and Amcinonide
Hydrochlorothiazide
Betamethasone benzoate
Minoxidil Betamethasone dipropionate
Pindolol Betamethasone valerate
Prazosin hydrochloride
Clobetasol propionate
Alseroxylon Clocortolone pivalate
Deserpidine Desonide
Rauwolfia Serpentina
Desoximetasone
Reserpine Dexamethasone sodium
Sodium nitroprusside
phosphate
Trimethaphan camsylate
Diflorasone diacetate
Amyl Nitrite Fluocinolone acetonide
Erythrityl tetranitrate
Oxitriptan
Isosorbide dinitrate
Carbidopa and Levodopa
Nitroglycerin Disulfiram
Pentaerythritol tetranitrate
Methyldopate
Indomethacin sodium
HCl
Morphine sulfate Glyceryl trinitrate
Hydromorphone Nitroglycerin absorbed on
Oxymorphone lactose
Methadone Octyl nitrite
Meperidine Sodium nitrite
Levorphanol Clonitrate
Codeine phosphate
Erythrityl tetranitrate
Pentazocine Mannitol hexanitrate
Nalbuphine Pentaerythritol tetranitrate
Butorphanol Pentrintrol
Steroids Triethanolamine trinitrate
Nonsteroidal Trolnitrate phosphate
anti-inflammatory agents
(triethanolamine trinitrate

Disease modifying
diphosphate)
anti-rheumatoid drugs
Amphetamines
Salicylates Pilocarpine
Ibuprofen Morphine
Fenoprofen L-DOPA
Naproxen Epinephrine
Piroxicam Nabilone
Tolmetin Isoproterenol
Indomethacin Catacholamines
Sulindac Metaproterenol
Meclofenamate Prostaglandins
Fentanyl

DETD In a presently preferred embodiment, the tolerance-inducing drug is isosorbide dinitrate (ISDN) or metabolites thereof, such as isosorbide 2-mononitrate (IS-2-MN) or isosorbide 5-mononitrate (IS-5-MN). Isosorbide dinitrate is a **vasodilator** which can be used to relieve the pain associated with angina pectoris, for the prevention of angina, in hypertension, for relaxation of involuntary muscles of blood vessels mainly arteries and arterioles, for increasing the flow of blood therein, and for increasing oxygenation from **vasodilation**, mainly for increasing the supply of oxygen to the heart.

DETD The drug delivery devices described herein can be utilized to deliver drugs for either prophylactic and/or therapeutic treatments. In therapeutic applications, the drug is administered to a patient already suffering from a disease in an amount sufficient to cure or at least partially arrest the symptoms of the disease and its complications. An amount adequate to accomplish this is defined as the "therapeutically effective amount or dose" or the "therapeutic plasma level". An amount below the therapeutically effective amount or dose or therapeutic plasma level is termed the "sub-therapeutically effective amount or dose" or the "sub-therapeutic plasma level". Amounts effective for this use will depend on the severity and course of the disease, previous therapy, the patient's health status and response to the drugs, and the judgment of the treating physician. See, e.g., American Medical Association (1992) Drug Evaluations Subscriptions; and Physicians' Desk Reference, 46th Ed. For example, in the case of angina pectoris, a therapeutically effective amount or dose of a **vasodilator**, such as isosorbide dinitrate, is an amount sufficient to relieve the pain associated with angina pectoris, to relax the involuntary muscles of blood vessels and to increase the flow of blood therein, or to increase oxygenation from **vasodilation**, thus increasing the supply of oxygen to the heart.

DETD For the 180.degree. adhesion test, about one inch was peeled back from a 1-inch wide strip of laminate prepared as described above. The laminate was slowly peeled apart for a distance of 6 to 61/2 inches and lowered adhesive side down directly onto a clean stainless steel panel in such a manner that one end touched first and the rest of the length followed smoothly to avoid trapping air bubbles and forming **wrinkles**. The rubber-covered steel roller was immediately drawn over the strip, without application of any additional pressure, lengthwise, without stopping, once from each direction, at a rate of about 2 inches/second. The laminate was allowed to stand undisturbed for 20.+-.5 minutes. The tab end was freed from the panel and gripped in the upper jaw of a tensile machine and the panel gripped in the lower jaw so that the strip was peeled off the panel at 180 degrees. The tensile machine was operated so that the jaws traveled at 12 inches/minutes. After 1 inch of the strip had been peeled off, the force was recorded and read at least five times at equally spaced intervals between 2 and 8 inches, averaged, and recorded as the 180.degree. release strength. Detailed procedures can be obtained from Dow Corning Corporation, Midland, Mich., as CTM 0964A.

AB A transdermal delivery system for the modulated administration of drugs

is described. The drug delivery device comprises a backing; a drug reservoir containing the drug, a plasticizer-type enhancer, a solvent-type enhancer, and optionally, a gelling agent; a non-rate-controlling membrane; and an adhesive layer containing a plasticizer-type enhancer. This drug delivery system is particularly useful for the administration of tolerance-inducing drugs, for example, **vasodilators**, such as isosorbide dinitrate.

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L21 ANSWER 3 OF 42 USPATFULL

SUMM In the final, remodeling phase (stage III), the previously constructed and randomly organized matrix is remodeled into an organized structure which is highly cross-linked and aligned to maximize mechanical strength. Natural skin **wrinkles** (relaxed skin tension lines) which align themselves in the direction of mechanical tension and become permanent on the face over time are a common manifestation of this control process. With hypertrophic scars and keloids, the biosynthetic phase continues longer than necessary to repair the wound. In order to maintain nutrient supply in these scars, vascular in-growth occurs, resulting in a large, highly vascularized scar which is unsightly and can be disabling.

SUMM Collagenolytic enzymes have been obtained following cell and organ culture from a wide range of tissues from animal species in which collagen is present. In general, these enzymes have a number of fundamental properties in common; they all have neutral pH optima; they are not stored within the cell, but, rather, appear to be secreted either in an inactive form or bound to inhibitors. FIG. 2 summarizes schematically the fundamental aspects of this enzyme and its mode of action. They appear to be zinc metalloenzymes requiring **calcium** , and are not inhibited by agents that block serine or sulphydryl-type proteinases. They are inhibited by kelating agents such as EDTA., 1.10-o-phenanthroline, and cysteine, which may inactivate zinc and perhaps other metals required for enzymatic activity and the zinc in the latent enzyme can be replaced by other divalent cations such as Co, Mn, Mg, and Cu. Nearly all the collagenases studied so far have a molecular mass that ranges from 25,000 to 60,000 daltons. The enzymes are usually present in a latent or inactive form. In some instances they seem to be associated with the presence of a zymogen, but in most cases are bound to an inhibitory protein component that can be removed to form the active enzyme; this step is accompanied by a decrease in molecular weight. Although proteolytic enzymes have been mostly used for activation, some latent collagenases can be activated by nonproteolytic agents, such as cheotropic salts or organic mercurial compounds, suggesting that the collagenase and inhibitors, though forming a tight complex, might not be peptide linked as in a proenzyme.

SUMM U.S. Pat. No. 5,132,119, incorporated herein by reference, has disclosed that **calcium** antagonists in various forms can drive the cells toward extracellular degradation instead of biosynthesis in the tissue culture environment. **Calcium** antagonists appear to influence cells to assume a more spherical shape, a result illustrated in FIG. 3. These fibroblasts will concomitantly change their metabolic status from one of synthesis to one of degradation. They also produce considerably more collagenase than the same cell in a more spread configuration, indicating that agents which depolymerize cytocellular proteins inhibit collagen synthesis and accelerate the activity of collagenase. Thus, the factors which control fibroblast shape also control the dynamic balance between extracellular matrix and degradation.

SUMM An additional aspect of the present invention contemplates a method for improving the appearance and size of scars by covering the scar with a thermal insulating material wherein the material contains a therapeutically effective amount of a medicament. In a preferred embodiment, the medicament is a **calcium** antagonist. More preferably, the **calcium** antagonist is a **calcium** inhibitor, a Protein Kinase C inhibitor, or a **calcium** transport blocker. Preferably, the **calcium** transport blocker is selected from the group consisting of verapamil, **nifedipine** , nicardipine, nimodipine, **diltiazem**, cobalt chloride and nickel chloride. Alternatively, the **calcium** transport blocker

is selected from the group consisting of phenylalkylamine compounds, benzothiazepine compounds and biologically compatible polyvalent salts. Still more preferably, the calmodulin inhibitor is trifluoperazine or tamoxifen.

DRWD FIG. 3 depicts the changes in collagen cell shape brought about by treatment with **calcium** antagonists and directly leading to collagen degradation.

DRWD FIG. 5a is a graphic representation of the effects of hydroxyurea (7.9 mM), antimycin A (1.0 .mu.M), and **nifedipine** (100 .mu.M) on the rate of proline incorporation;

DRWD FIG. 8 is a graphic representation of the incorporation of .sup.3 H-proline into FPCM extracellular matrix. FPCM bathed in fructose were untreated (control) or treated with 100 .mu.M **diltiazem**.

DRWD FIG. 10 is a graphic representation of the effect of the **calcium** antagonist verapamil (50 .mu.M) on release of Lucifer Yellow CH from human dermal fibroblasts in monolayer culture. Retardation of exocytosis is demonstrated.

DETD The present invention thus contemplates a method for improving the appearance and size of scars by covering the scar with a thermal insulating material containing a **calcium** antagonist. Preferably, the **calcium** antagonist is a **calcium** inhibitor, a Protein Kinase C inhibitor, or a **calcium** transport blocker. Preferably, a **calcium** transport blocker is verapamil, **nifedipine**, nicardipine, nimodipine, **diltiazem**, cobalt chloride or nickel chloride. Alternatively, the **calcium** transport blocker is a phenylalkylamine compound, a benzothiazepine compound or a biologically compatible polyvalent salt. Still more preferably, the calmodulin inhibitor is trifluoperazine or tamoxifen.

DETD **Calcium** antagonists, as used herein, are compounds which interfere with **calcium** transport within a cell or block/inhibit one or more events involved in the **calcium** cascade. Several classes of **calcium** antagonists include calmodulin inhibitors, Protein Kinase C inhibitors and **calcium** transport blockers. Calmodulin inhibitors prevent the binding of **calcium** to calmodulin, thereby interrupting intracellular signal transduction, including activation of Protein Kinase C, the next event in the **calcium** cascade. Compounds that inhibit Protein Kinase C or other downstream events can be used. Calmodulin inhibitors include phenothiazines, such as trifluoperazine and tamoxifen (also Protein Kinase C inhibitors). **Calcium** transport blockers, also called **calcium** entry antagonists, **calcium** channel antagonists or **calcium** channel blockers, block the action of **calcium** channels, which are regions of cell membranes that facilitate the transport and secretion of fluids and electrolytes such as **calcium** into the cell [Rasmussen, It. N. E. J. Med. 314: 1094-1101 (1986)]. Compounds included in this class are phenylalkylamine compounds, such as verapamil; polyvalent ionic salts that physically block the **calcium** channels, such as nickel chloride, cobalt chloride and other biologically acceptable salts of these; hydropyridine compounds, such as **nifedipine**; and benzothiazepine compounds, such as **diltiazem**. Other compounds that affect the secondary messenger pathways in cellular signal transduction may have the same or similar effect as **calcium** antagonists on cell shape and tissue remodeling.

DETD A method of the present invention utilizes the discovery that **calcium** antagonists, which interfere with **calcium** metabolism or transport across the cell membrane, can inhibit exocytosis in fibroblast cells; can retard biosynthesis of collagen and sulfated glycosaminoglycans (GAG); can be used to decrease the collagen content of the extracellular matrix; and can also stimulate increased collagenase activity, leading to softening of the scar tissue. These features work together to control wound scar production; by minimizing,

preventing or reversing the scarring process, depending upon the course of the disease or type of wound treated.

DETD Exocytosis, a process involved in cellular secretion of protein, is but one mechanism affected by **calcium** antagonist treatment. During secretion, vesicles that contain sorted and concentrated protein pinch off from the Golgi apparatus and move toward the cell membrane at the leading edge of the cell, where they fuse with the cell membrane and release protein into the extracellular space. This process of fusion and release is known as exocytosis and is one of the essential steps in secretion of extracellular matrix macro-molecules (such as glycosaminoglycans, collagen and elastin). Many diseases and disorders are characterized by excessive biosynthesis or secretion. For example, hypertrophic wound healing disorders are characterized by over-secretion of protein and collagen. This over-production is one factor which contributes to excessive scarring or keloid formation.

DETD **Calcium** antagonists also regulate cell shape. As described in detail in the Examples, fibroblasts that have been treated with a **calcium** antagonist became more rounded than untreated fibroblasts. The treated cells were tested for viability and were found to have intact cell membranes which are indicative of viable cells. The observation that treated fibroblast cells become altered was correlated with changes in cell programming from a biosynthetic mode (mechanism normally undertaken by untreated fibroblasts) to a degradative mode. It is believed that this change toward matrix degradation, mediated by cell shape changes, plays a roll in controlling wound scar production. Thus, other compounds can be studied for their ability to regulate (up regulate or down regulate) fibroblast biosynthesis by observing their interaction with **calcium** antagonists.

DETD In one embodiment, wound scar content can be minimized by incorporating an effective amount of a **calcium** antagonist into a thermal insulating material, such as a hydrogel, covering a hypertrophic wound site. A scar is covered with a hydrogel containing the **calcium** antagonist alone, or in combination with a protein synthesis inhibitor (e.g., steroid). Treatment of the wound site by covering with a hydrogel containing the **calcium** antagonist, with or without the steroid, should continue for a period of time sufficient to minimize the wound area. Suitable **calcium** antagonists include, but are not limited to phenylalkylamine compounds, such as verapamil; biologically acceptable polyvalent salts, such as cobalt chloride and nickel chloride; hydropyridine compounds, such as **nifedipine** nicardipine and nimodipine; and phenothiazines, such as trifluoperazine and tamoxifen which are examples of calmodulin inhibitors.

DETD The amount of **calcium** antagonist which can be effectively administered is dependent upon the type of **calcium** antagonist used and the scar site to be treated, as well as the nature of the hydrogel or other thermal insulating material used. In an ideal embodiment, the amount is adjusted accordingly depending upon the response observed. Exemplary threshold effective amounts of verapamil and **nifedipine** are approximately 10 .mu.M and 1 mM, respectively. Steroid which can be used include, but are not limited to; corticosteroids and glucocorticosteroids, such as triamcinolone acetonide (also known as KENALOG.TM.), and Vitamin E (.alpha.-tocopherol) (Ehrlich et al. 1972, Ann. Surg. 75:235). The amount of steroid which can be effectively administered depends upon the type of steroid used, and the nature of the hydrogel or other thermal insulating material used. The effects of **calcium** antagonist treatment, with and without steroids, on various types of wound scars are illustrated in the Examples.

DETD Hydropyridine compounds such as **nifedipine** are relatively insoluble in aqueous solution. Due to their insolubility, it is advantageous to solubilize the drug in a carrier which facilitates its incorporation into a hydrogel.

DETD Depolymerization of cycloskeletal proteins leading to alteration of the cell shape and matrix degradation can be regulated using a methods of

this invention. Secondary to this, the invention can be used to regulate and block exocytosis. In particular, fibroblasts are contacted with an effective amount of a **calcium** antagonist incorporated into a hydrogel or other suitable material, an amount sufficient to degrade the matrix and retard exocytosis to a desired degree.

DETD Effect Of **Calcium** Antagonists On Biosynthesis

DETD The effect of **calcium** antagonists on protein and glycosaminoglycan (GAG) biosynthesis was measured in FPCMs under several conditions. The biosynthetic responses to **calcium** antagonism were studied in FPCMs cultured n DMEM supplemented with either 5.5 .mu.M glucose or 5.5 .mu.M fructose. Both were studied because energy metabolism of cultured fibroblasts is primarily anaerobic when the carbohydrate energy source is glucose and predominantly aerobic when the carbohydrate source is fructose [Thilly, W. G., Mammalian Cell Technology, Chapter 5, Butterworth Publishers, Boston, (1986)]. In vivo fibroblasts are, however, believed to derive their energy primarily through aerobic glycolysis.

DETD The drugs used to antagonize **calcium** channels were verapamil, **nifedipine**, cobalt chloride and trifluoperazine. Control studies were performed to test the metabolic state of the cells in the FPCM. The effect of hydroxyurea and antimycin A, a drug which blocks oxidative phosphorylation, on biosynthesis was measured in FPCMs cultured in fructose or glucose.

DETD When the effect of the carbohydrate source on the rate of biosynthesis of protein and glycosaminoglycan was examined in FPCMs bathed in DMEM/0.5 mM cold proline, no difference was observed between control FPCMs in glucose or fructose (FIG. 6). There was a dose-dependent effect of verapamil on protein incorporation. However, the biosynthetic response to **calcium** channel blockers was observed to depend on the type of **calcium** antagonist used and whether the carbohydrate source was glucose or fructose.

DETD In equimolar concentrations, **nifedipine** caused a larger reduction of .sup.3 -proline incorporation than verapamil. As for verapamil, **nifedipine** at 100 .mu.M concentration had no effect on GAG biosynthesis. When the medium was supplemented with fructose, **nifedipine** at 10 and 100 .mu.M reduced both proline and sulfate incorporation by 60%. In contrast, **nifedipine** at 1 .mu.M had no effect on either proline or sulfate incorporation. In a series of 3-week-old FPCMs, twelve hours' incubation in 100 .mu.M **nifedipine** caused complete digestion of the matrix.

DETD When glucose was used as the carbohydrate source, verapamil at 100 .mu.M concentration was also found to retard the incorporation of .sup.3 H-proline into the extracellular matrix about 50%. In fact, fibroblasts appeared to be more sensitive to verapamil when glucose was used as the carbohydrate source. The incorporation of .sup.3 H-proline in the samples treated with 1 and 10 .mu.M verapamil was about 20% less than that of the control. Different concentrations of verapamil also had no effect on sulfated GAG biosynthesis. In summary, it was observed that verapamil an **nifedipine** at 100 .mu.M each reduced .sup.3 H-proline incorporation by almost 50-60 in the tissue equivalent.

DETD The rates of .sup.3 H-proline and sulfated glycosaminoglycan incorporation in fibroblast populated collagen matrices bathed in DMEM/5.5 .mu.M glucose or fructose and a **calcium** antagonist are shown in Table 1.

DETD The effects of other **calcium** antagonists have been studied. Preliminary data showed that 100 .mu.M ditiazen reduced .sup.3 H-proline incorporation by about 30% in the connective tissue equivalent. See FIG. 8. Trifluoperazine has also been studied.

DETD To determine if the rate of fluid phase exocytosis was modulated by **calcium** antagonists, the rate of exocytosis in human fibroblasts was measured using the rate of release of Lucifer yellow labeled dextran (LYD, M. W. 10,000) (Molecular Probes Inc., Portland, Oreg.), from vesicles in the cytoplasm of human foreskin fibroblasts. The LYD was loaded into cells by fluid phase pinocytosis (endocytosis) in the

absence of serum. The intracellular location and transport of the dye was monitored under control and experimental conditions using video image analysis.

DETD Exocytosis was observed to proceed at a near constant rate over a six hour period of observation in human dermal fibroblasts in monolayer culture (FIG. 7b). The rate of exocytosis of Lucifer yellow dextran was found to be sensitive to plasma membrane **calcium** channel function. Both verapamil (10 .mu.M) and **nifedipine** (100 .mu.M) were found to significantly retard exocytosis over a six hour period in these cells (FIG. 6, Tables II and III). These results clearly demonstrate that exocytosis in human fibroblasts can be regulated. In FIG. 7b, the controls are represented by the squares. Table II and III show the retardation of exocytosis in human dermal fibroblasts by **calcium** channel blockers, verapamil (50 .mu.M) and **nifedipine** (1 .mu.M), respectively.

DETD TABLE III

Effect Of **Nifedipine** On Exocytosis Response

Stimulus	Exposure Time (Hours)	Average Normalized Intensity	Standard Error	p Value
Control	0	.58	.029	
	4	.54	.029	
	6	.36	.015	
Nifedipine:				
1 .mu.M	4	.58	.029	p > 0.05
	6	.49	.047	p < 0.03

DETD Study On Cell Shape Changes Caused By **Calcium** Antagonists

DETD Light and electron microscopy studies indicated that verapamil caused the cells to adopt a more rounded shape than controls. These rounded cells were tested for viability by staining the cells with 0.01% trypan blue for 5 minutes. Most of the cells were not stained with trypan blue indicating that the cell membranes were intact and cells remain viable. Again, alteration of cell shape correlates with the change in cell programming from biosynthetic mode to a degradative mode. Based upon this observation, it is hypothesized that **calcium** channel blockers drive the cells toward matrix degradation, perhaps mediated by cell shape changes.

CLM What is claimed is:

2. The method of claim 1 wherein the medicament is a **calcium** antagonist.

3. The method of claim 2 wherein the **calcium** antagonist is a calmodulin inhibitor, a Protein Kinase C inhibitor, or a **calcium** transport blocker.

4. The method of claim 3 wherein the **calcium** transport blocker is verapamil, **nifedipine**, nicardipine, nimodipine, **diltiazem**, cobalt chloride or nickel chloride.

5. The method of claim 3 wherein the **calcium** transport blocker is a phenylalkylamine compound, a benzothiazepine compound, or a biologically compatible polyvalent salts.

9. The method of claim 8 wherein the medicament is a **calcium** antagonist.

10. The method of claim 9 wherein the **calcium** antagonist is a calmodulin inhibitor, a Protein Kinase C inhibitor, or a **calcium** transport blocker.

11. The method of claim 10 wherein the **calcium** transport blocker is verapamil, **nifedipine**, nicardipine, nimodipine, **diltiazem**, cobalt chloride or nickel chloride.

12. The method of claim 10 wherein the **calcium** transport blocker is a phenylalkylamine compound, a benzothiazepine compound, or a biologically compatible polyvalent salts.

AB A method for improving the size and appearance of a scar associated with a fibromatosis, a keloid, or a hypertrophic wound healing disorder comprises stimulating collagenase activity in the scar. Preferably, stimulating collagenase activity is accomplished by covering said scar with a thermal insulating material that elevates the surface temperature of the scar. Further disclosed is a method for improving the size and appearance of a scar comprises covering said scar with a thermal insulating material that elevates the surface temperature of the scar and that contains a therapeutically effective amount of a medicament.

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L72 ANSWER 24 OF 24 USPATFULL on STN

SUMM "If a tensional stress is imposed on connective tissue over a long period, the **fibroblasts** which make up most of its bulk orient themselves along the lines of stress and begin to multiply more rapidly. They produce more collagen, the fibrous infrastructure of connective tissue. The extra fibers reduce the elasticity of the tissue. As collagen is fairly resistant to enzyme breakdown, these changes tend to be irreversible. The extra fibers take up space in the connective tissue of the muscle, and begin to encroach on the space normally occupied by nerves, blood and lymph vessels. As a result of this crowding, the tissue loses its elasticity and sometimes becomes painful when the muscle is set to work. The required work might then be attempted via another region of tissue, and the useful life of that region would be limited."

SUMM In general, the benefits of connective tissue loosening and improved blood circulation are endless. Even appearance is improved such as a healthier appearing skin. Scalp and/or **facial** massage with the technique herein described is considered by some subjects to have provided **wrinkle** reduction and other cosmetic improvements. The technique, when applied to subjects suffering from various forms of arthritis, has reportedly resulted in improvement in range of motion and comfort in performing tasks which were otherwise painful. These and other yet unknown benefits are likely to result from connective tissue loosening and improved blood circulation generated by the present invention. Another less obvious application may be the treatment of problems that develop in space travel. Recent reports on the hazards of space travel suggest that inhibited red blood cell production and reduced **muscle** resistance may be a serious problem with persons spending long periods in a condition of weightlessness. The present invention or improvements or modifications thereto may provide the answer.

ACCESSION NUMBER:	96:5394 USPATFULL
TITLE:	Method and device for loosening connective tissue and stimulating blood circulation
INVENTOR(S):	Pitzen, Sylvester A., Phoenix, AZ, United States
PATENT ASSIGNEE(S):	Sono Therapy Institute, Inc., Phoenix, AZ, United States (U.S. corporation)

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et al, Proc. Soc. Exp. Biol. Med., 169: 445 (1982)).
Moreover, increased collagen production by cultured **fibroblasts** derived from keloids persists throughout their in vitro life span. It appears that once keloid **fibroblasts** responsible for keloids overcome entropy, they do not revert to normal even after being removed from the lesions and placed in culture. No significant differences in DNA content or cellularity were observed in keloids compared with normal dermis, although that matter is still debated. These data suggest that each **fibroblast** within a keloid is producing excessive collagen, as opposed to an increased number of **fibroblasts** each producing a normal amount of collagen.

SUMM Reasons have been proposed (Cohen et al, Plastic Surgery, Vol. 1, General Principles, pp. 732-747 (1990)) as to why excessive collagen production occurs and persists in abnormal scars as well as in the **fibroblasts** derived from those lesions. As mentioned, it may be that excessive collagen producing **fibroblasts** are selected by the wound environment and that this selection results in excessive collagen production and deposition by **fibroblasts** in the lesions. This hypothesis is supported by several studies. In one such study (Hunt et al, Am. J. Surg., 135: 328 (1978)) it was reported that increased hypoxia was noted in early animal wounds and that hypoxia stimulates macrophages, in turn, to stimulate **fibroblast** collagen production. There is reason to believe that keloids are hypoxic, because microvascular occlusion is frequent and some portions of keloids are relatively avascular. Moreover, increased lactate, increased histamine and decreased pH (Cohen et al, Plastic Surgery, Vol. 1, General Principles, pp. 732-747 (1990)) are characteristics of abnormal scars that conceivably could create a "stressed" environment selecting **fibroblasts** that are high collagen producers.

SUMM The hypoxic-selectivity hypothesis is substantiated by several reports demonstrating that heterogenous populations of **fibroblasts** with particular biochemical characteristics can be isolated from normal tissue. Perhaps certain kinds of **fibroblasts** predominate in abnormal wounds and either (1) fail to respond to regulatory signals ending increased collagen production during early wound healing or (2) are selected and proliferate more abundantly in the "stress" environment of the early wound. There is evidence that keloid-derived **fibroblasts**, which can be isolated in vivo, are a selected subset of normal dermal **fibroblasts** that occur more abundantly in abnormal scars. For example, **fibroblasts** grown out of keloid tissue produce increased extracellular matrix components in vitro (Diegelmann et al, Proc. Soc. Exp. Biol. Med., 169: 445 (1982)), and demonstrate a differential response to hydrocortisone and histamine (Russell et al, J. Cell. Physiol., 93: 389 (1977) and Topol et al, Plast. Reconstr. Surg., 68: 227 (1981)) compared with normal **fibroblasts**. Recent studies have shown that keloid-derived **fibroblasts** have reduced growth factor requirements. Such studies indicate that the "type" of **fibroblast** in abnormal scars is different from **fibroblasts** in normal dermis. However, such "abnormal" cells are morphologically identical to normal **fibroblasts** and grow at the same rate (Diegelmann et al, Proc. Soc. Exp. Biol. Med., 169: 445 (1982)).

SUMM The question of collagen type abnormalities in abnormal scars is raised frequently. After injury to normal skin, the ratio of Type III to Type I collagen increases and then subsides to a normal value of about 17 to 20% of total collagen as wound healing progresses. It is known that Type III collagen is increased in granulation tissue and in hypertrophic scars, but Type III collagen appears to occur in a normal amount in keloids. It has been reported that keloid **fibroblasts** overproduce Type I collagen, while Type III collagen expression remains unchanged. This finding also suggests that keloids are dissimilar to

early wounds. This is surprising because other parameters such as elevated water content, increased soluble collagen, and increased histamine indicate that mature keloids resemble early wounds. It is possible that abnormal collagen types have not been found because of limitations of typing methodology. More sophisticated typing methods may identify abnormal ratios and types of collagen in abnormal scars.

SUMM There has never been a clear histologic difference between keloids and hypertrophic scars. Over two decades ago, one study differentiated keloids from hypertrophic scars on the basis that keloids appear to contain bundles of collagen with focal proliferation or nodules and increased quantities of mucopolysaccharides. Another more recent study reviewed the literature on histology of abnormal scars and reported that collagen in both keloids and hypertrophic scars is organized into discrete nodules, frequently (but not always) obliterating the rete pegs in the papillary dermis of the lesions. Whereas the collagen in normal dermis is arranged in discrete fascicles, separated by considerable interstitial space, the collagen nodules in keloids and in hypertrophic scars appear avascular and unidirectional, and are aligned in a "highly stressed" configuration. The origin and significance of characteristic collagen nodules in abnormal scars are unknown at the present time. While **myofibroblasts** have been found in keloids and hypertrophic scars, their role in abnormal scar formation remains obscure. The relationship between the histology and the pathophysiology of these lesions remains an enigma.

SUMM Steroids have been shown to decrease the size of keloids in a number of clinical studies and decrease collagen synthesis in vitro studies, specifically performed on keloid and normal dermal **fibroblasts** . Surprisingly, collagen production as measured by propyl hydroxylase was not decreased in lesions previously treated with triamcinolone. Nevertheless, triamcinolone acetonide is the steroid of choice for intralesional treatment of keloids. Moderately insoluble intralesional triamcinolone acetonide has been claimed to be effective in reducing the size of the keloids and hypertrophic scars. It has also been suggested that keloid resorption after steroid treatment may, in part, be due to steroid enhancement of collagenase activity. There is data to suggest that corticosteroids not only inhibit protein synthesis, but also enhance collagenase activity.

SUMM The use of ionizing radiation as a means of treating keloids was first attempted in the early 1900's and thereafter with questionable success. Radiation non-selectively destroys collagen-producing **fibroblasts** in lesions as well as in surrounding connective tissue and cells--a significant drawback to its use. Even when combined with surgery and chemotherapy, radiation does not appear to provide an effective, preventive modality for abnormal scar or keloid formation. Although there are no known reports of radiation-induced carcinoma following treatment of abnormal scars with radiation, caution is always recommended because of this possibility.

SUMM Manipulation of the type of suture material and experiments with different suture techniques have been proposed as methods of obviating possible abnormal scar formation. There are no data to suggest that the type of suture material or surgical closure technique is in any way involved in the etiology of abnormal scar (Cohen et al, Plastic Surgery, Vol. 1, General Principles, pp. 732-747 (1990)). However, tension and lines of relaxed skin tension may be related to hypertrophic scar formation. Wound closure parallel to the lines of relaxed skin tension usually produces **fine-line** scars, whereas wound closure perpendicular to the lines of relaxed skin tension tend to form hypertrophic scars.

SUMM For several years highly concentrated human fibrin sealant has been

recommended for bowl anastomoses, liver repair, and spleen surgery (Yaita et al, Japanese J. Surg., 5: 56-63 (1975) and Orda et al, J. Surg. Res. 17: 365-374 (1974)), mainly due to its reliable hemostatic effect. The intra-abdominal use of fibrin sealant is still a matter of debate. Fibrin sealant is a method of sealing peritoneal surfaces with physiological agents. The sealant gives a smooth surface and prevents exudates and bleeding. On the other hand, the sealant acts as a substrate for **fibroblast** proliferation (Staindl et al, Arch. Otorhinolaryngol., 233: 105-166 (1981) and Hedelin et al, Scand. J. Plast. Reconstr. Surg., 17: 179-181 (1983)) and thus may promote adhesions. One study (Lindenberg et al, Ann. Chir. Gynaecol., 73: 11-13 (1984)) showed a protective effect when fibrin sealant was used to cover sutured parietal peritoneum in rats. In a second study on rats (Lindenberg et al, Acta Chir. Scand., 151: 525-527 (1985)), it was demonstrated that adhesion formation was inversely correlated with the thickness and lifetime of the fibrin clot. However, even with a thin layer of fibrin, adhesion formation was significantly greater than in an untreated control group. Furthermore, fibrin sealant has been successfully used in humans (Baumann et al Geburtsh Frauenheilkd, 46: 234-236 (1986)) and in animals (Gauwerky et al, Human Reprod., 3: 327-330 (1988)) for tubal surgery, with no increase in adhesion formation observed. Since the fibrin clot is an optimal substrate for the ingrowth of **fibroblasts** and consequent collagen synthesis and fibrosis, adhesion promoting qualities may result.

SUMM Results from a recently completed study (Golan et al, Int. J. Fert., 36: 317-320 (1991)) agree with (Lacey, Ann. Surg., 90: 281 (1930)) experimental observations made over fifty years ago. Comparing amniotic fluid to saline controls, no inhibitory effect of the amniotic fluid was demonstrated. The effect of amniotic fluid and the control saline solutions on **fibroblast** proliferation was examined in vitro using fibroblastic cell cultures. No direct effect on **fibroblast** proliferation was found. The conclusion of the aforementioned recent study (Golan et al, Int. J. Fert., 36: 317-320 (1991)) was that it was not the direct effect of the spillage of amniotic fluid that inhibits adhesion formation after the performance of cesarean sections.

SUMM It is known that following motor nerve severance **muscle** atrophies and is eventually replaced by fibrous tissue. Detailed investigations into the denervation process have ben summarized in studies. Denervated **muscle** or **muscle** graft can become innervated by one of three mechanisms: surgical neurorhaphy; implantation of nerve directly into the **muscle**; and sprouting of nerves from adjacent normal **muscle**, i.e., muscular neurotization. In clinical situations in which a nerve is not available, the latter mechanism is predominant. The phenomenon of one **muscle** innervating another has been observed clinically. One study noted that following a pharyngeal flap there is often reanimation of surrounding soft palate musculature. In failed neurotization it is likely that fascia is a barrier to the reinnervation between **muscles**. Most reports of free **muscle** grafts in humans have occurred in reconstruction of **facial** and anal **muscles**, neither of which have fascial coverings.

SUMM It is not known how long a **muscle** can exist before irreversible atrophy and fibrosis makes attempts to introduce neural innervation unsuccessful. It is likely that different **muscles** undergo atrophy at different rates. It has been observed that intrinsic **muscles** of the hand atrophy within months after denervation, yet successful reinnervation of **facial muscles** has been reported one year after **facial** nerve injury. There seems little question, however, that the longer the period of denervation, the more unsuccessful is the reinnervation process. Alterations in the normal physiologic length of **muscle** adversely influence

function. It is well known from clinical experience that after an unrecognized tendon laceration, reduction of the resting length of the **muscle** results in atrophy, fibrosis, and loss of normal elasticity within a few weeks of injury. If insufficient resting tension exists, the **muscle** fibers decrease in cross sectional area and shorten in length, thus limiting motor function and strength. It is also true that increasing the length of a **muscle** and applying excessive stretch results in fibrosis and loss of contractile force.

SUMM The protein isolate is preferably administered in combination with one or more compounds selected from the group consisting essentially of inducers of cell proliferation, inducers of cell differentiation, antibiotics, and anti-inflammatory agents. The protein isolate has a gene encoding the scar inhibitory protein, said gene being isolated and inserted directly into cells so that their synthesized product can be used in an endocrine, paracrine, or autocrine fashion to inhibit mesenchymal stem cell differentiation into scar **fibroblasts** and subsequent scar tissue formation.

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DETD Based on review of the prior art studies and investigation, it is believed that the Scar Inhibitory Factor (SIF) of this invention is a binding protein isolate with the potential to either bind directly to a "scar" morphogenetic protein; acting as a competitive inhibitor, to bind to a cell; surface receptor for the scar morphogenetic protein; or to bind to a closely associated cell surface receptor that can block the scar morphogenetic protein receptor. Scar morphogenetic protein is what induces the differentiation of resident mesenchymal stem cells into "scar" **fibroblasts**. These scar **fibroblasts** are subsequently involved in the deposition of extracellular matrix material forming normal scars, hypertrophic scars, keloids, and/or fibrous adhesions.

DETD SIF is comprised of one or more heretofore unidentified non-collagenous proteins comprising basement membranes. Intact basement membranes, located between epithelia/endothelia/parenchyma and the underlying connective tissues, provide a supportive structure and effectively form a mechanical barrier to inhibit **fibroblast** infiltration and scar formation. SIF assists the mechanical action of the basement membrane by forming a chemical barrier, radiating from the basement membrane, to competitively inhibit the action of scar morphogenetic protein. SIF thereby assists inhibiting scar **fibroblast** formation and their subsequent infiltration through the basement membrane, thus preventing scar formation.

DETD As discussed below in both in vitro and in vivo model systems, SIF is neither a cytotoxic agent of stem cells, a growth inhibitor of stem cells, nor does it affect the differentiation potential of the mesenchymal stem cells into other tissue phenotypes, i.e., muscle, cartilage, bone, fact, and/or structural **fibroblasts**. SIF's only discovered activity to date appears to be the inhibition of differentiation of mesenchymal stem cells into scar **fibroblasts** , thereby allowing normal differentiation to occur.

DETD The EDTA extracts were pooled and concentrated into three aliquots of 300 ml each by Amicon.TM. ultrafiltration with a YM10 membrane. Each 300 ml EDTA aliquot was washed with five liters of double distilled water. Precipitates formed at each step were removed by centrifugation until

only those proteins soluble in cold distilled water remain. This portion of the extract was lyophilized and constituted a water soluble **fibroblast** inhibitory protein isolate.

DETD By the third day of treatment, the control cultures with MMP demonstrated two types of responses. One response shown in FIG. 3B consisted of two morphologically distinct cell types, stellate-shaped cells and spindle-shaped cells (similar in appearance, respectively, to mesenchymal cells and **fibroblasts** as described by Young et al in 1992. In FIG. 3B, the cells labelled SP are the spindle-shaped (fibroblastic) cells. It is also important to note the absence of any myotubes within the culture.

DETD Furthermore, the results shown in FIGS. 4A-4H also dramatically demonstrate the differences between cultured mesenchymal cells and the cells of living animals treated with and without SIF. In vivo treatment without MMP and SIF results in scar tissue as shown in FIG. 4A. Similarly, FIG. 4B shows that in vitro treatment with only MMP results in **fibroblasts**. However, FIG. 4C exhibits an absence of scarring.

ACCESSION NUMBER: 1998:131607 USPATFULL
TITLE: Pluripotent mesenchymal stem cells and methods of use thereof
INVENTOR(S): Young, Henry E., Macon, GA, United States
Lucas, Paul A., Poughkeepsie, NY, United States
PATENT ASSIGNEE(S): MorphoGen Pharmaceuticals, Inc., New York, NY, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5827735		19981027
APPLICATION INFO.:	US 1996-650420		19960520 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1995-393453, filed on 23 Feb 1995 which is a continuation of Ser. No. US 1992-901860, filed on 22 Jun 1992, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Saunders, David
LEGAL REPRESENTATIVE: Klauber & Jackson
NUMBER OF CLAIMS: 17
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 15 Drawing Figure(s); 4 Drawing Page(s)
LINE COUNT: 2119

L72 ANSWER 19 OF 24 USPATFULL on STN

TI Compositions and methods for treating **wrinkles** and/or **fine lines** of the skin

AB Compositions which contain an agonist substance of one or a number of receptors associated with a chlorine channel are useful for slackening and/or relaxing cutaneous tissue, and in particular for the purpose of treating **wrinkles** and **fine lines** of the skin. Such compositions can be administered topically or by injection. Preferred agonists include glycine, serine, taurine, .beta.-alanine, N-(benzyloxycarbonyl)glycine (Z-glycine), gamma-aminobutyric acid (GABA), isoguvacine, isonipecotic acid, 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyrid-3(2H)-one, benzodiazepines, steroids, and barbiturates. The composition can additionally contain a retinoid and/or a hydroxy acid.

SUMM The present invention relates to the use of substances which are agonists of a receptor associated with a chlorine channel in a cosmetic and/or dermatological composition, in particular for the purpose of treating **wrinkles** and **fine lines** of the skin, and to cosmetic and/or dermatological compositions which contain such a substance.

SUMM Women, and indeed even men, are currently inclined to wish to appear young for as long as possible and consequently are looking to soften the signs of ageing of the skin, which are reflected in particular by **wrinkles** and **fine lines**. In this respect, advertising and fashion present products intended to retain a radiant and **wrinkle**-free skin, these being the signs of young skin, for as long as possible, all the more so since physical appearance has an effect on mental attitude and/or on morale. It is consequently important to feel physically and spiritually young.

SUMM Until now, **wrinkles** and **fine lines** have been treated using cosmetic products containing active agents which act on the skin, for example by moisturizing it or by improving its cell renewal or alternatively by promoting the synthesis of collagen of which the cutaneous tissue is composed. However, to date, it is not known to act on **wrinkles** by involving the muscle components present in the skin.

SUMM It is known that the platysma **muscles** of the face are under the control of the motor nerve afferent activity of the **facial** nerve and that, moreover, the interlobular septa of the hypoderm contain within them fibers which constitute a striated **muscle** tissue (panniculus carnosus). Moreover, it is also known that a subpopulation of **fibroblasts** of the dermis, known as **myofibroblasts** , has characteristics in common with the **muscle** tissue.

SUMM The Applicants have observed, in certain pathological and therapeutic situations, the role played, as regards the **wrinkles** of the face, by the nerves controlling all this **muscle** tissue. Thus, in attacks on the **facial** nerve, in which transmission of the nerve impulse is interrupted and/or weakened, a paralysis of the **muscles** of the face is witnessed in the area of innervation. This **facial** paralysis is reflected, among other clinical indications, by an alleviation in, indeed disappearance of, the **wrinkles**.

SUMM On the other hand, in muscle hypercontraction conditions of the face, the Applicants have observed an accentuation in the **wrinkles** of the face. Moreover, an accentuation in the **wrinkles** of the face has also been observed in muscle hypertonia conditions of Parkinson's disease and side-effects induced by neuroleptics.

SUMM Moreover, it has been shown that botulinus toxin, originally used for treating spasms, could have an effect on muscle spasticity conditions

(see A. Blitzer et al., Arch. Otolaryngol. Head Neck Surg., vol 119, pages 1018 to 1022 (1993)) and on the **wrinkles** of the glabella, which are intersuperciliary **wrinkles** (see J. D. Carruters et al., J. Dermatol. Surg. Oncol., vol. 18, pages 17 to 21 (1992)). It is consequently possible, by pharmacological action, to have an effect on the nerve component of **wrinkles**. Botulinus toxin acts directly at the level of the neuro-muscular junction by blocking the action of acetylcholine on muscular tenseness.

SUMM However, to date no completely suitable compositions or methods are available for treating **wrinkles** and/or **fine lines** of the skin. Thus, there remains a need for methods and compositions effective for treating **wrinkles** and/or **fine lines** of the skin.

SUMM Accordingly, it is one object of the present invention to provide novel compositions for treating **wrinkles** and/or **fine lines** of the skin.

SUMM It is another object of the present invention to provide novel methods for treating **wrinkles** and/or **fine lines** of the skin.

SUMM These and other objects, which will become apparent during the following detailed description, have been achieved by the inventors' discovery that contractile muscle fibers, which are under the direct control of the neuromotor impulse, play an essential role in the pathogenesis of **wrinkles** and that suppression of the neuromotor impulse alleviates not only **wrinkles** but also **fine lines** and also has a "smoothing" effect on the cutaneous microrelief. It has also been found that cutaneous tissues contain receptors associated with chlorine channels, something which, until now, had not been envisaged. It has thus been found that it is possible to act on these channels in order to slacken or relax these tissues and thus to lessen **wrinkles** and **fine lines**.

SUMM Until now, a connection between the chlorine channels of nerve fibers of the peripheral cutaneous nervous system and **wrinkles** had never been established, nor had it been found that it was possible to treat **wrinkles** by acting on chlorine channels by activation of the receptors which are found in or in the neighborhood of these channels. Substances which can activate the receptors of chlorine channels and thus lead to the entry of chloride into cells are known as agonist substances.

SUMM In another aspect, the present invention provides injectable cosmetic or dermatological compositions, for the purpose of lessening **wrinkles** and/or **fine lines**, which contain at least one agonist substance of at least one receptor associated with at least one chlorine channel present in cutaneous tissue for relaxing and/or slackening cutaneous tissue. In this context, the term "injectable" means suitable for injection into tissue, and in particular in **wrinkles**.

SUMM The present invention additionally provides topical cosmetic or dermatological compositions for the purpose of lessening **wrinkles** and/or **fine lines** which contain at least one agonist substance of at least one receptor associated with at least one chlorine channel of at least one cutaneous afferent nerve pathway, except glycine and gamma-butyric acid, for relaxing and/or slackening cutaneous tissue.

SUMM Another aspect of the present invention is a method for the cosmetic treatment of **wrinkles** and/or **fine lines** in

humans by injecting a composition containing at least one agonist substance of at least one receptor associated with at least one chlorine channel present in cutaneous tissue.

SUMM It is certainly known to use GABA and glycine in combination with other active agents for combating ageing of the skin but, until now, their action in relaxing and slackening cutaneous tissues for the purpose of treating **wrinkles** was not known. The generally known actions are inhibition of elastase, the effect on collagen, and cell renewal.

SUMM Mention may especially be made, among the active agents which the compositions of the invention can contain, of active agents having an effect on the treatment of **wrinkles** or of **fine lines** and in particular of keratolytic active agents. The term "keratolytic active agent" is understood to mean an active agent having desquamative, exfoliative or scrubbing properties or an active agent capable of softening the corneal layer.

SUMM Mention may in particular be made, among these active agents having an effect on the treatment of **wrinkles** or **fine lines** which the compositions of the invention can contain, of hydroxy acids and retinoids.

DETD The lotion obtained has an effect on **wrinkles** during repeated use (twice daily application for one month).

DETD The gel obtained has an effect on **wrinkles**. It can be applied daily, morning and evening, for one month.

DETD A white oily cream is obtained which has an effect on **wrinkles** and **fine lines** and which can be applied daily.

CLM What is claimed is:

7. A method for lessening **wrinkles** or **fine lines**, by relaxing or slackening cutaneous tissue comprising topically applying a **wrinkle** or **fine line** lessening effective amount of at least one agonist substance of at least one receptor associated with at least one chlorine channel of at least one cutaneous afferent nerve pathway, with the proviso that said agonist is a benzodiazepine, a steroid or a barbiturate.

13. A method for lessening **wrinkles** or **fine lines**, comprising administering by injection a cosmetic or dermatological composition, said composition comprising at least one agonist substance of at least one receptor associated with at least one chlorine channel present in cutaneous tissue wherein said agonist substance is a benzodiazepine, steroid or barbiturate.

25. A method for the cosmetic treatment of **wrinkles** or **fine lines** in humans, comprising injecting a composition comprising at least one agonist substance of at least one receptor associated with at least one chlorine channel present in cutaneous tissue wherein said agonist substance is a benzodiazepine, steroid or barbiturate.

ACCESSION NUMBER:	1999:136709 USPATFULL		
TITLE:	Compositions and methods for treating **wrinkles** and/or **fine lines** of the skin		
INVENTOR(S):	De Lacharriere, Olivier, Paris, France Breton, Lionel, Versailles, France		
PATENT ASSIGNEE(S):	L'Oreal, Paris, France (non-U.S. corporation)		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5976559		19991102
APPLICATION INFO.:	US 1998-50959		19980331 (9)

RELATED APPLN. INFO.:	Continuation of Ser. No. US 1995-538119, filed on 2 Oct 1995, now patented, Pat. No. US 5869068

	NUMBER	DATE
PRIORITY INFORMATION:	FR 1994-11742	19940930
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	Granted	
PRIMARY EXAMINER:	Venkat, Jyothsna	

SUMM The use of ionizing radiation as a means of treating keloids was first attempted in the early 1900's and thereafter with questionable success. Radiation non-selectively destroys collagen-producing **fibroblasts** in lesions as well as in surrounding connective tissue and cells--a significant drawback to its use. Even when combined with surgery and chemotherapy, radiation does not appear to provide an effective, preventive modality for abnormal scar or keloid formation. Although there are no known reports of radiation-induced carcinoma following treatment of abnormal scars with radiation, caution is always recommended because of this possibility.

SUMM Manipulation of the type of suture material and experiments with different suture techniques have been proposed as methods of obviating possible abnormal scar formation. There are no data to suggest that the type of suture material or surgical closure technique is in any way involved in the etiology of abnormal scar (Cohen et al, Plastic Surgery, Vol. 1, General Principles, pp. 732-747 (1990)). However, tension and lines of relaxed skin tension may be related to hypertrophic scar formation. Wound closure parallel to the lines of relaxed skin tension usually produces **fine-line** scars, whereas wound closure perpendicular to the lines of relaxed skin tension tend to form hypertrophic scars.

SUMM For several years highly concentrated human fibrin sealant has been recommended for bowl anastomoses, liver repair, and spleen surgery (Yaita et al, Japanese J. Surg., 5: 56-63 (1975) and Orda et al, J. Surg. Res. 17: 365-374 (1974)), mainly due to its reliable hemostatic effect. The intra-abdominal use of fibrin sealant is still a matter of debate. Fibrin sealant is a method of sealing peritoneal surfaces with physiological agents. The sealant gives a smooth surface and prevents exudates and bleeding. On the other hand, the sealant acts as a substrate for **fibroblast** proliferation (Staindl et al, Arch. Otorhinolaryngol., 233: 105-166 (1981) and Hedelin et al, Scand. J. Plast. Reconstr. Surg., 17: 179-181 (1983)) and thus may promote adhesions. One study (Lindenberg et al, Ann. Chir. Gynaecol., 73: 11-13 (1984)) showed a protective effect when fibrin sealant was used to cover sutured parietal peritoneum in rats. In a second study on rats (Lindenberg et al, Acta Chir. Scand., 151: 525-527 (1985)), it was demonstrated that adhesion formation was inversely correlated with the thickness and lifetime of the fibrin clot. However, even with a thin layer of fibrin, adhesion formation was significantly greater than in an untreated control group. Furthermore, fibrin sealant has been successfully used in humans (Baumann et al Geburtsh Frauenheilkd, 46: 234-236 (1986)) and in animals (Gauwerky et al, Human Reprod., 3: 327-330 (1988)) for tubal surgery, with no increase in adhesion formation observed. Since the fibrin clot is an optimal substrate for the ingrowth of **fibroblasts** and consequent collagen synthesis and fibrosis, adhesion promoting qualities may result.

SUMM Results from a recently completed study (Golan et al, Int. J. Fert., 36: 317-320 (1991)) agree with (Lacey, Ann. Surg., 90: 281 (1930)) experimental observations made over fifty years ago. Comparing amniotic fluid to saline controls, no inhibitory effect of the amniotic fluid was demonstrated. The effect of amniotic fluid and the control saline solutions on **fibroblast** proliferation was examined in vitro using fibroblastic cell cultures. No direct effect on **fibroblast** proliferation was found. The conclusion of the aforementioned recent study (Golan et al, Int. J. Fert., 36: 317-320 (1991)) was that it was not the direct effect of the spillage of amniotic fluid that inhibits adhesion formation after the performance of cesarean sections.

SUMM It is known that following motor nerve severance **muscle**

atrophies and is eventually replaced by fibrous tissue. Detailed investigations into the denervation process have ben summarized in studies. Denervated **muscle** or **muscle** graft can become innervated by one of three mechanisms: surgical neurorhaphy; implantation of nerve directly into the **muscle**; and sprouting of nerves from adjacent normal **muscle**, i.e., muscular neurotization. In clinical situations in which a nerve is not available, the latter mechanism is predominant. The phenomenon of one **muscle** innervating another has been observed clinically. One study noted that following a pharyngeal flap there is often reanimation of surrounding soft palate musculature. In failed neurotization it is likely that fascia is a barrier to the reinnervation between **muscles**. Most reports of free **muscle** grafts in humans have occurred in reconstruction of **facial** and anal **muscles**, neither of which have fascial coverings.

SUMM It is not known how long a **muscle** can exist before irreversible atrophy and fibrosis makes attempts to introduce neural innervation unsuccessful. It is likely that different **muscles** undergo atrophy at different rates. It has been observed that intrinsic **muscles** of the hand atrophy within months after denervation, yet successful reinnervation of **facial muscles** has been reported one year after **facial** nerve injury. There seems little question, however, that the longer the period of denervation, the more unsuccessful is the reinnervation process. Alterations in the normal physiologic length of **muscle** adversely influence function. It is well known from clinical experience that after an unrecognized tendon laceration, reduction of the resting length of the **muscle** results in atrophy, fibrosis, and loss of normal elasticity within a few weeks of injury. If insufficient resting tension exists, the **muscle** fibers decrease in cross sectional area and shorten in length, thus limiting motor function and strength. It is also true that increasing the length of a **muscle** and applying excessive stretch results in fibrosis and loss of contractile force.

SUMM The protein isolate is preferably administered in combination with one or more compounds selected from the group consisting essentially of inducers of cell proliferation, inducers of cell differentiation, antibiotics, and anti-inflammatory agents. The protein isolate has a gene encoding the scar inhibitory protein, said gene being isolated and inserted directly into cells so that their synthesized product can be used in an endocrine, paracrine, or autocrine fashion to inhibit mesenchymal stem cell differentiation into scar **fibroblasts** and subsequent scar tissue formation.

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DETD Based on review of the prior art studies and investigation, it is believed that the Scar Inhibitory Factor (SIF) of this invention is a binding protein isolate with the potential to either bind directly to a "scar" morphogenetic protein; acting as a competitive inhibitor, to bind to a cell; surface receptor for the scar morphogenetic protein; or to bind to a closely associated cell surface receptor that can block the scar morphogenetic protein receptor. Scar morphogenetic protein is what induces the differentiation of resident mesenchymal stem cells into "scar" **fibroblasts**. These scar **fibroblasts** are

subsequently involved in the deposition of extracellular matrix material forming normal scars, hypertrophic scars, keloids, and/or fibrous adhesions.

DETD SIF is comprised of one or more heretofore unidentified non-collagenous proteins comprising basement membranes. Intact basement membranes, located between epithelia/endothelia/parenchyma and the underlying connective tissues, provide a supportive structure and effectively form a mechanical barrier to inhibit **fibroblast** infiltration and scar formation. SIF assists the mechanical action of the basement membrane by forming a chemical barrier, radiating from the basement membrane, to competitively inhibit the action of scar morphogenetic protein. SIF thereby assists inhibiting scar **fibroblast** formation and their subsequent infiltration through the basement membrane, thus preventing scar formation.

DETD As discussed below in both in vitro and in vivo model systems, SIF is neither a cytotoxic agent of stem cells, a growth inhibitor of stem cells, nor does it affect the differentiation potential of the mesenchymal stem cells into other tissue phenotypes, i.e., muscle, cartilage, bone, fact, and/or structural **fibroblasts**. SIF's only discovered activity to date appears to be the inhibition of differentiation of mesenchymal stem cells into scar **fibroblasts**, thereby allowing normal differentiation to occur.

DETD The EDTA extracts were pooled and concentrated into three aliquots of 300 ml each by Amicon.TM. ultrafiltration with a YM10 membrane. Each 300 ml EDTA aliquot was washed with five liters of double distilled water. Precipitates formed at each step were removed by centrifugation until only those proteins soluble in cold distilled water remain. This portion of the extract was lyophilized and constituted a water soluble **fibroblast** inhibitory protein isolate.

DETD By the third day of treatment, the control cultures with MMP demonstrated two types of responses. One response shown in FIG. 3B consisted of two morphologically distinct cell types, stellate-shaped cells and spindle-shaped cells (similar in appearance, respectively, to mesenchymal cells and **fibroblasts** as described by Young et al in 1992. In FIG. 3B, the cells labelled SP are the spindle-shaped (fibroblastic) cells. It is also important to note the absence of any myotubes within the culture.

DETD Furthermore, the results shown in FIGS. 4A-4H also dramatically demonstrate the differences between cultured mesenchymal cells and the cells of living animals treated with and without SIF. In vivo treatment without MMP and SIF results in scar tissue as shown in FIG. 4A. Similarly, FIG. 4B shows that in vitro treatment with only MMP results in **fibroblasts**. However, FIG. 4C exhibits an absence of scarring.

ACCESSION NUMBER: 1998:131607 USPATFULL

TITLE: Pluripotent mesenchymal stem cells and methods of use thereof

INVENTOR(S): Young, Henry E., Macon, GA, United States
Lucas, Paul A., Poughkeepsie, NY, United States

PATENT ASSIGNEE(S): MorphoGen Pharmaceuticals, Inc., New York, NY, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5827735		19981027
APPLICATION INFO.:	US 1996-650420		19960520 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1995-393453, filed on 23 Feb 1995 which is a continuation of Ser. No. US 1992-901860, filed on 22 Jun 1992, now abandoned

DOCUMENT TYPE: Utility

FILE SEGMENT: Granted

PRIMARY EXAMINER: Saunders, David

LEGAL REPRESENTATIVE: Klauber & Jackson

NUMBER OF CLAIMS: 17

EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 15 Drawing Figure(s); 4 Drawing Page(s)
LINE COUNT: 2119
CAS INDEXING IS AVAILABLE FOR THIS PATENT.